Sperm Ultrastructure of *Xenos vesparum* (Rossi) and its Significance in the Taxonomy and Phylogeny of Strepsiptera (Insecta)

Marcella CARCUPINO, Giuseppe PROFILI*, Jeyaraney KATHIRITHAMBY** & Massimo MAZZINI**

*Dipartimento di Scienze Ambientali,
Università della Tuscia, I - 01100 Viterbo, Italy.
** Department of Zoology,
University of Oxford, South Parks Road, OX1 3PS Oxford, England.

ABSTRACT

Sperm ultrastructure of the strepsipteran *Xenos vesparum* was studied by scanning and transmission electron microscopy. The data obtained were compared with those of other strepsipteran species, namely *Xenos moutoni*, *Elenchus tenuicornis*, *E. japonicus* and *Halictophagus chilensis*. From the spermatological point of view, Strepsiptera appear to form a uniform group in which the mature sperm consist of: an elongated head with a monolayered acrosome and a nucleus characterized by an eccentric portion of uncondensed chromatin; and a long tail consisting of a 9+9+2 axoneme flanked by two mitochondrial derivatives of equal size. The spermatozoon of *X. vesparum*, however, shows some species-specific characters such as a longer nucleus which is entirely occupied by condensed chromatin, and two U-shaped mitochondrial derivatives which are devoid of paracrystalline material. These results confirm the sperm morphology as valuable taxonomic character, even if it is not yet sufficient to clarify the controversial phylogenetic affinities of Strepsiptera.

RÉSUMÉ

Ultrastructure du spermatozoïde de *Xenos vesparum* (Rossi) et sa signification pour la taxonomie et la phylogénie des Strepsiptères (Insecta).

L'ultrastructure du spermatozoïde du Strepsiptère *Xenos vesparum* a été étudiée en microscopie électronique à transmission et à balayage. Les données obtenues ont été comparées avec celles des autres Strepsiptères *Xenos moutoni*, *Elenchus tenuicornis*, *E. japonicus* et *Halictophagus chilensis*. D'un point de vue spermatologique, les Strepsiptères semblent former un groupe homogène dans lequel le spermatozoïde mûr consiste en une tête allongée comprenant un acrosome à une seule couche et un noyau caractérisé par une portion excentrique de chromatine non condensée, et une longue queue formée d'un axonème 9+9+2 flanqué de deux dérivés mitochondriaux de taille égale. Toutefois, le spermatozoïde de *X. vesparum* montre quelques caractères spécifiques tels qu'un noyau plus long qui est entièrement occupé par la chromatine condensée, et deux dérivés mitochondriaux en forme de U qui sont dépourvus de matériel paracristallin. Ces résultats confirment la valeur de la morphologie des spermatozoïdes comme caractère taxonomique, même s'ils ne sont pas actuellement suffisant pour éclaircir la position phylogénétique controversée des Strepsiptères.

CARCUPINO, M., PROFILI, G., KATHIRITHAMBY, J., & MAZZINI, M., 1995. — Sperm ultrastructure of *Xenos vesparum* (Rossi) and its significance in the taxonomy and phylogeny of Strepsiptera (Insecta). *In*: JAMIESON, B. G. M., AUSIO, J., & JUSTINE, J.-L. (eds), Advances in Spermatozoal Phylogeny and Taxonomy. *Mém. Mus. nat. Hist. nat.*, **166**: 291-296. Paris ISBN: 2-85653-225-X.

Strepsiptera constitute a small cosmopolitan order of entomophagous parasitic insects which are characterized by extreme sexual dimorphism with free-living, short-lived males and permanently endoparasitic neotenic females (except in the family Mengenillidae).

The ordinal status of the Strepsiptera is still debated. Even up to the present day, several workers, mainly coleopterists, place Strepsiptera as a family (Stylopidae) in the order Coleoptera (beetles) [2, 9, 10, 20] or as a sister group of the Coleoptera [13]. Using classical morphological characters, KINZELBACH [17] reported the plesiomorphies and apomorphies of Strepsiptera. A large number of the apomorphic characters are adaptations to an endoparasitic life; the only apomorphic character uniting Strepsiptera and Coleoptera was found to be the use of the hind wings as the main organ for flight, with consequent specialization of the metathorax for this function.

Recently, KRISTENSEN [18] divided the holometabolic insects into two groups, one consisting of Megaloptera, Raphidioptera, Neuroptera and Coleoptera, the other of Panorpoidea and Hymenoptera, whereas the position of Strepsiptera remained unsolved.

More recently, on the basis of morphological and genetic analysis, a close relationships between Strepsiptera and Diptera has been suggested [13, 21].

Examination of other characters, such as the morphology of the sperm, could provide useful contributions to the phylogeny of Strepsiptera. JAMIESON [11] has coined the term spermiocladistic for the use of sperm ultrastructure in the reconstruction of the phylogeny. There are many examples of spermatological characters (such as the organization of the acrosomal complex and the tail) useful for phylogenetic and evolutionary reconstructions in many animal groups, particularly in insects [for a review see 3, 4, 11].

Descriptions of sperm ultrastructure and spermiogenesis of Strepsiptera have been reported. BACCETTI [5] was the first to describe the ultrastructure of the sperm tail of *Xenos vesparum* Rossi (Stylopidae). Later [8, 15, 16, 19], the sperm organization was studied in four species, namely *Xenos moutoni* De Buysson (Stylopidae), *Elenchus tenuicornis* Kirby, *E. japonicus* Esaki & Hashimoto (Elenchidae) and *Halictophagus chilensis* Hofmann (Halictophagidae). More recently, CARCUPINO *et al.* [9] have examined spermiogenesis in *E. tenuicornis*, while AFZELIUS & DALLAI [1], using a new fixation technique, studied the tail organization in *Stylops* sp.

The present paper aims to provide additional information on the mature sperm and the male reproductive system in *X. vesparum*, and to compare this with all the previous data on the sperm structure in Strepsiptera, in order to contribute to a better understanding of the phylogenetic position of this peculiar Insect order.

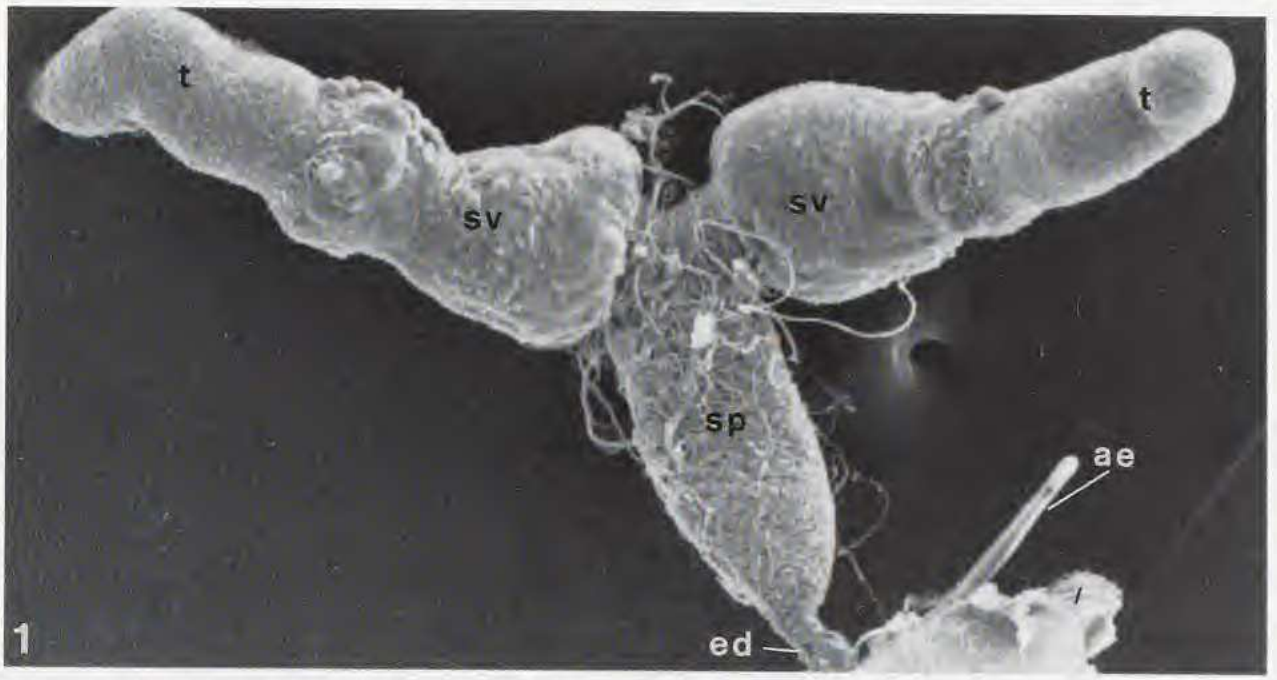
MATERIALS AND METHODS

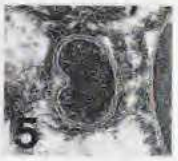
Stylopized wasps *Polistes dominilus* (Christ) were collected in the vicinity of Tübingen (Germany) and brought back to the laboratory. The internal reproductive systems were dissected from free-living males of *X. vesparum* and processed for scanning (SEM) and transmission (TEM) electron microscopy.

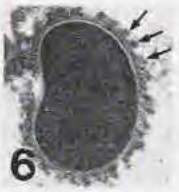
Scanning electron microscopy. Specimens were fixed in Karnovsky's fixative [12] for 2 h at 4° C, rinsed overnight in 0.1 M cacodylate buffer at pH 7.2, post-fixed in similarly buffered 1% osmium tetroxide, dehydrated in a graded ethanol series, dried with the critical point method, gold coated and observed with a JEOL JSM 5200 electron microscope.

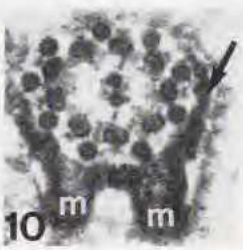
FIG. 1. — SEM micrograph of the internal reproductive system of adult male *Xenos vesparum* showing aedeagus (ae), ejaculatory duct (ed), sperm pump (sp), seminal vesicles (sv), and testes (t). x 215

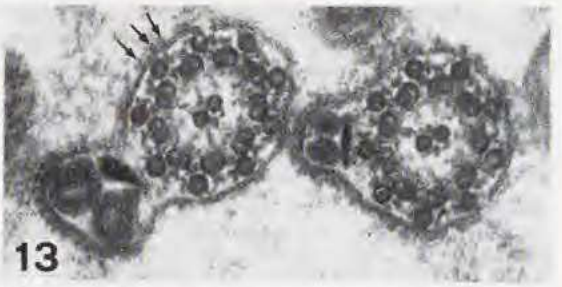
FIGS 2-15. — Longitudinal (2, 8) and cross-sections (3-7, 9-15) at different levels of the spermatozoon of *X. vesparum*. Observe the brush-like glycocalyx at each level of the sperm surface (arrows). **2:** Longitudinal section of the sperm head. x 14 400. **3:** Cross-section of the acrosome. x 90 000. **4-7:** Cross-sections at different level of the kidney-shaped nucleus, from the apex (4) to the base (7). x 90 000. **8-10:** Longitudinal (8) and cross-section (9,10) of the neck region of the sperm tail showing the centriolar area with mitochondrial derivatives (m) characterized by crescent-like appendages (arrows) encircling the axoneme (ax). 8, x 54 000, 9-10, x 90 000. **11-15:** Cross-sections at different levels of the tail with mitochondrial derivatives (m) reducing in size and axoneme (ax) progressively disorganized. x 90 000.











Transmission electron microscopy. Specimens were fixed and dehydrated as above, then embedded in Epon-Araldite mixture. Thin sections were cut in a Reichert Ultracut and a LKB Nova ultramicrotomes, stained with uranyl acetate and lead citrate and observed with a JEOL JEM 1200 EX II electron microscope.

RESULTS

The reproductive system of *X. vesparum* (Fig. 1) includes a pair of elongated testes which open into two large pear-shaped seminal vesicles. The seminal vesicles in turn open separately into a single large muscular sperm pump via two thick vasa deferentia. A single ejaculatory duct emerges from the posterior portion of the sperm pump and terminates in the aedeagus (Fig. 1).

The morphology of the mature sperm is similar to that of the other species of *Xenos* previously examined [19]. The sperm is filiform with an elongated head and a long tail in which three different regions, namely neck region, principal piece and end piece, can be recognized (Figs 2-15).

The head, about 8.5 µm long, is occupied by a monolayered acrosome and a nucleus (Figs 2, 3). In cross-section, the nucleus, which is narrower in the apical portion, is kidney-shaped and appears entirely occupied by compact and electron-dense chromatin (Figs 4-7).

The tail has a simple organization with the axoneme flanked by two long mitochondrial derivatives (Figs 8-13). The axoneme has the common 9+9+2 pattern in which nine accessory tubules, nine microtubular doublets with two dynein arms, and two central microtubules can be recognized (Figs 11-13). The mitochondrial derivatives have typical cristae looking like a series of lamellae which are regularly spaced and aligned at right-angles to one side of the major axis of the derivatives (Fig. 8). No paracrystalline structure seems to be present in the mitochondrial matrix.

The neck region shows a bilocular head-tail junction in which the centriolar region with the beginning of the axoneme and the mitochondrial derivatives is located (Fig. 8). Cross-sections of this region show that the mitochondrial derivatives have a U-shaped appearance with a crescent-shaped appendage encircling the axoneme on each side (Figs 9, 10). These appendages progressively reduce in size until they disappear. Along the principal piece, the axoneme is always flanked by two U-shaped mitochondrial derivatives from which it is separated by a conspicuous membranous sheath (Figs 11, 12). Proceeding toward the endpiece, the mitochondrial derivatives reduce in size and the axoneme becomes disorganized (Figs 13-15). Along the endpiece, axonemal disorganization occurs with the disappearance of the nine accessory tubules first (Fig. 14), then of the two central microtubules, and later of the nine peripheral doublets (Fig. 15).

The entire surface of the sperm, from the acrosome to the endpiece, is covered by a thin brush-like glycocalyx (Figs 3-7, 9-15).

DISCUSSION

The spermatozoon of *Xenos vesparum* is similar to that of other species of Strepsiptera examined previously. Except for a few peculiarities observed in the sperm ultrastructure of each species examined [8, 15, 16, 19], such as different shape of the acrosome, nucleus and mitochondrial derivatives, Strepsiptera were considered a uniform group. The mature sperm is filiform and consists of a monolayered acrosome at the tip of an elongated nucleus which has a portion of uncondensed chromatin in an eccentric position, and a bilocular head-tail junction followed by a 9+9+2 axoneme flanked by two mitochondrial derivatives of equal size.

The mature sperm of *X. vesparum* shows some characters similar to that of *X. moutoni* [19], such as U-shaped mitochondrial derivatives and sperm surface covered by a brush-like glycocalyx, which could be considered as generic characteristics. However, the spermatozoon of *X. vesparum* also shows several species-specific characters. The sperm nucleus is about twice as long as that of the other species examined and lacks the internal portion of uncondensed chromatin. The mitochondrial derivatives have an unusual appearance. Like the typical mitochondrial derivatives, they have cristae with the appearance of a series of lamellae regularly

spaced and aligned orthogonally to the major axis of the sperm, but they lack the paracrystalline material. Mitochondria without any sign of crystallization were also reported in *Stylops* sp. [1].

These data confirm that the morphology of the spermatozoon has real value in providing taxonomic characters, and that the spermatozoon of Strepsiptera does not resemble that of Coleoptera. Coleoptera sperm resemble those of Strepsiptera in having the tail with a 9+9+2 axoneme flanked by two mitochondrial derivatives but differ in having a three layered acrosomal complex (the acrosomal vesicle is located between the periacrosomal cap and the subacrosomal material) and two accessory bodies (for a review see [6, 11]). However, the phylogenetic affinities of Strepsiptera remain unsolved.

Recently, AFZELIUS & DALLAI [1], studying insect sperm tails fixed with a glutaraldehyde-tannic acid mixture, reported that Strepsiptera sperm deviate in several important respects from those of other components of the neuropteroid superorder (Megaloptera, Raphidioptera, Neuroptera and Coleoptera). In particular, the incomplete kidney-shaped accessory axonemal tubules, the lack of the intratubular material and of the accessory bodies distinguished the strepsipteran spermatozoa from those of any other insect groups [1]. The sperm tail of *X. vesparum*, however, as well as those of the other strepsipteran species previously examined [5, 8, 15, 16, 19] shows normal circular accessory tubules. It is not easy to discriminate if this difference is related to specific characteristics or to the new fixative, or to different stages of sperm maturation. In fact, by using the glutaraldehyde-tannic acid mixture, the axonemal structures are better resolved, and therefore the kidney shape might represent the real shape of the strepsipteran accessory tubules. However, the kidney-shaped accessory tubules showed in *Stylops* sp. [1] belong to an immature sperm as demonstrated by the presence of microtubules flanking the mitochondria (see micrograph number 8 in [1]). On this basis, it could be hypothesized that the strepsipteran accessory tubules are kidney-shaped during the differentiation stages and become circular in cross-section at the end of maturation. A survey of mature spermatozoon of *Stylops* sp. could clarify this matter.

Whether these unusual features of the strepsipteran sperm reflect an adaptation to an endoparasitic life, or an isolated systematic position (or both) is still unclear. In fact, related to the endoparasitic life are a very simple female reproductive system [14] and a peculiar mode of insemination and fertilization to which the organization of the sperm could be also related. Further light might be thrown on this problem by a study of sperm morphology in the Mengenillidae, a primitive strepsipteran family in which both males and females are free-living.

REFERENCES

1. AFZELIUS, B. A. & DALLAI, R., 1994. — Characteristics of the flagellar axoneme in Neuroptera, Coleoptera and Strepsiptera. *Journal of Morphology*, **219**: 15-20.
2. ARNETT, R. H., 1968. — *The Beetles of the United States (a Manual for Identification)*. The American Entomological Institute: 1-112.
3. BACCETTI, B., 1972. — Insect sperm cells. *Advances in Insect Physiology*, **9**: 315-397.
4. BACCETTI, B., 1979. — The evolution of the acrosomal complex. *In*: D. W. FAWCETT & J. M. BEDFORD, *The spermatozoon*. Baltimore & München, Urban & Schwarzenberg: 305-329.
5. BACCETTI, B., 1989. — The spermatozoon of Strepsiptera and its value in the systematic position of the group. *Journal of Submicroscopic Cytology and Pathology*, **21**: 397-398.
6. BURRINI, A. G., MAGNANO, L., MAGNANO, A. R., SCALA, C. & BACCETTI, B., 1987. — Spermatozoa and phylogeny in Curculionoidea (Coleoptera). *International Journal of Insect Morphology and Embryology*, **17**: 1-50.
7. CARCUPINO, M., KATHIRITHAMBY, J. & MAZZINI, M., 1994. — Spermiogenesis in *Elenchus tenuicornis* (Kirby) (Strepsiptera: Elenchidae). *Atti XVII Congresso nazionale di Entomologia, Udine 13-18 Giugno*: 343-346.
8. CARCUPINO, M., MAZZINI, M., OLMI, M. & KATHIRITHAMBY, J., 1993. — The spermatozoon of *Halictophagus chilensis* Hofmann (Strepsiptera, Halictophagidae). *Bolletino di Zoologia*, **60**: 361-365.
9. CROWSON, R. A., 1960. — The phylogeny of Coleoptera. *Annual Review of Entomology*, **5**: 111-134.
10. CROWSON, R. A., 1981. — *The Biology of Coleoptera*. New York, Academic Press: 1-802.

11. JAMIESON, B. G., 1987. — *The Ultrastructure and Phylogeny of Insect Spermatozoa.* Cambridge, Cambridge University Press: 1-320.
12. KARNOVSKY, M. J., 1965. — A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, **27**: 137A-138A.
13. KATHIRITHAMBY, J., 1989. — Review of the order Strepsiptera. *Systematic Entomology*, **14**: 41-92.
14. KATHIRITHAMBY, J., CARCUPINO, M., & MAZZINI, M. 1990. — Ovarian structure in the order Strepsiptera. *Frustula Entomologica*, **13**: 1-8.
15. KATHIRITHAMBY, J., CARCUPINO, M. & MAZZINI, M., 1992. — Ultrastructure of the spermatozoon of *Elenchus japonicus* and its bearing on the phylogeny of Strepsiptera. *Tissue and Cell*, **24**: 437-442.
16. KATHIRITHAMBY, J., CARCUPINO, M. & MAZZINI, M., 1993. — Comparative spermatology of four species of Strepsiptera and comparison with a species of primitive Coleoptera (Rhipiphoridae). *International Journal of Insect Morphology & Embryology*, **22**: 459-470.
17. KINZELBACH, R. K., 1971. — Morphologische Befunde an Fächerflüglern und ihre phylogenetische Bedeutung (Insecta: Strepsiptera). *Zoologica*, **41**: 1-256.
18. KRISTENSEN, N. P., 1989. — Insect phylogeny based on morphological evidences. *In*: B. FERNHOLM, K. BREMER & H. JÖRNVALL, *The Hierarchy of Life*. Amsterdam, Elsevier: 295-306.
19. MAZZINI, M., CARCUPINO, M. & KATHIRITHAMBY, J., 1991. — Fine structure of the spermatozoon of the strepsipteran *Xenos moutoni*. *Tissue and Cell*, **23**: 199-207.
20. ROSS, H. T., ROSS, A. C. & ROSS, J. R. P., 1984. — *A Textbook of Entomology, 4th Edition*. New York, Wiley: 1-666.
21. WHITING, M. F. & WHEELER, W. C., 1994. — Insect homeotic transformation. *Nature*, **368**: 696.